

Filovirus infections

Kelly L. Warfield, PhD; Emily M. Deal, BS; Sina Bavari, PhD

Many emerging and reemerging human pathogens are derived from animals or from animal tissues, waste, or products. A wide range of species including insects (eg, mosquitos), wild animals (eg, rodents, bats, and monkeys), draft animals (eg, horses and mules), and livestock animals (eg, swine, poultry, and cattle) have been implicated in transmission of various highly pathogenic infections, including parasitic, bacterial, and viral diseases, to humans. Among the most deadly emerging zoonotic diseases are viral hemorrhagic fevers, including those caused by the filoviruses, EBOV and MARV. The zoonotic potential of these viruses was identified at the time of their discovery during the first recognized filovirus outbreak that simultaneously occurred in Germany and Yugoslavia. During this 1967 outbreak, laboratory workers became infected following contact with blood and organs from MARV-infected African green monkeys that had been imported from Uganda. The focus of this review is the zoonotic nature of the filoviruses.

Filovirus Pathobiology

Ebola virus and MARV, members of the family *Filoviridae* in the order *Mononegavirales*, are highly pathogenic viruses that cause hemorrhagic fever and that require manipulation under restrictive biosafety level 4 conditions.¹ Only 1 species of MARV is known to date; however, 5 species of EBOV have emerged. These EBOV species include Zaire, Sudan, and Côte d'Ivoire viruses, which cause fatal infection in 40% to 90% of infected patients, and Reston, which causes asymptomatic infections in humans but is lethal to nonhuman primates. A potential fifth species that is named Bundibugyo was isolated in 2007.^{1,2}

From Integrated BioTherapeutics Inc, 20358 Seneca Meadows Pkwy, Germantown, MD 20876 (Warfield); the Department of Microbiology and Immunology, School of Medicine, Stanford University, Stanford, CA 94305 (Deal); and Target Identification and Translational Research, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702-5011 (Bavari).

Supported in part by the Defense Threat Reduction Agency, Joint Science Technology Office—Chemical Biological Program, and by the United States Army Medical Research and Materiel Command (Drs. Warfield and Bavari) or the National Institutes of Health, grant No. AI021362 (for Ms. Deal [as awarded to Dr. Harry Greenberg]).

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army.

The authors would like to thank Drs. Tom Larsen and Tony Alves for provision of photographs and Larry Ostby for assistance with map production.

Address correspondence to Dr. Warfield.

ABBREVIATIONS

EBOV	Ebola virus
FVLP	Filovirus-like particle
MARV	Marburg virus

In humans, hemorrhagic fever viruses, including the filoviruses, cause a nonspecific viral prodrome that includes fever, chills, headache, myalgia, and anorexia. Other nonspecific symptoms such as abdominal pain, sore throat, nausea, vomiting, cough, arthralgia, and diarrhea may subsequently develop, and patients are often dehydrated, apathetic, and disoriented when they present with disease. Clinical signs of filovirus infections in nonhuman primates are highly similar to the clinical features of filovirus hemorrhagic fever in humans. One of the more distinguishing signs of filovirus infection is a maculopapular rash on the trunk and limbs (Figure 1). Other hemorrhagic manifestations, including bleeding from the gastrointestinal and urogenital tracts, petechia, and hemorrhage from injection sites and mucous membranes, may develop during the peak of the illness (usually 5 to 10 days after onset of symptoms).

The differential diagnoses for filovirus infections are extensive and include malaria, cholera, influenza, typhoid fever, viral encephalitis, dengue fever, or other viral hemorrhagic fevers. Assessments of general clinicopathologic variables are not helpful for diagnosis, and outbreaks are typically only recognized after a large number of persons have been affected and national or international health authorities have been called for assistance. Mortality rates during filovirus outbreaks are generally quite high (as high as 90%), although this rate appears variable and depends on the location of the outbreak and the virus isolate involved.³⁻⁵ Although it is understood from evidence derived by use of animal models of filovirus-induced disease that virulence depends on the virus species, the relationship between geographic locations of outbreaks and mortality rates is not yet understood; among outbreaks, mortality rates could vary on the basis of virus species, differences in patient care between developed and developing countries, and general health of the affected population, along with many other variables. There are no approved treatments or vaccines for use in humans for EBOV or MARV infections.

Macrophages and dendritic cells appear to be early targets of the infection in vivo, and during in vitro studies,⁶⁻⁹ these cells supported high levels of virus replication. Consistent with their apparent early role in viral replica-

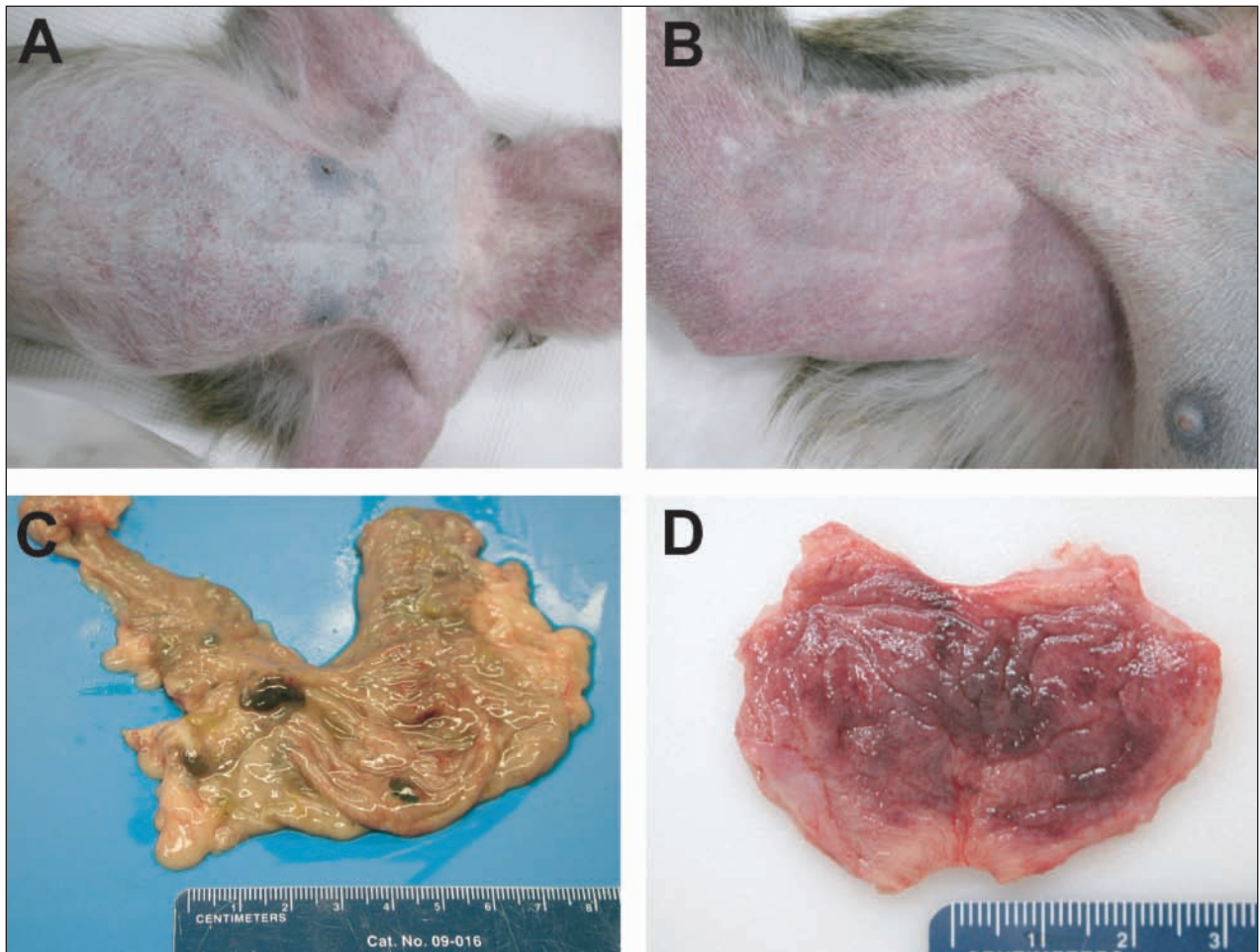


Figure 1—Photographs to illustrate clinical features that are typically associated with filovirus infection in humans and nonhuman primates. A—Ventral aspect of the cranial portion of the body of a filovirus-infected cynomolgus monkey. Notice the maculopapular rash on the trunk (head is to the right). B—Medial aspect of the upper portion of the right forelimb of a filovirus-infected cynomolgus monkey. The maculopapular rash frequently extends along the arms; the face and hind limbs are often affected. C—Portion of the gastrointestinal tract of an EBOV-infected macaque obtained at necropsy. Notice the multifocal petechial and ecchymotic hemorrhages with moderate mucosal congestion at the ileo-cecal junction. D—Portion of the urinary bladder (luminal aspect) of a MARV-infected macaque obtained at necropsy. Notice the diffuse mucosal hemorrhage and congestion.

tion, there is evidence that that these critical antigen-presenting cells do not function properly following infection and are somehow impaired by the filovirus infection.^{8,9} The virus is likely distributed rapidly through the body following the initial infection event (usually a percutaneous event involving blood contact), resulting in development of high concentrations of virus both in many organs (including liver, kidneys, spleen, and lungs) and in the cells that compose these organs.^{7,10} In most infections, the resultant tissue damage is irreparable, especially in the liver and lymphoid tissues. Endothelial cells are also infected by the filoviruses; however, the kinetics and role of their infection in filovirus pathogenesis are disputed.^{6,10–12} Lymphocytes, including natural killer cells, are not infected, but these cell types are severely affected during filovirus infection as a result of bystander apoptosis.^{13,14}

Epidemiology

The filoviruses were first identified following the 1967 outbreak in Germany and Yugoslavia among laboratory workers who became infected after processing

blood and tissues from MARV-infected monkeys imported from Uganda.^{15–17} More recent MARV cases or outbreaks occurred across Africa in countries including South Africa, Zimbabwe, Kenya, the Democratic Republic of the Congo, and Angola, with case fatality rates ranging from 20% in Germany in 1967 to > 80% in Angola during the outbreak in 2004 and 2005.¹⁷ Ebola virus was first identified during disease outbreaks that occurred in northern Zaire and southern Sudan in 1976; subsequently, infections with EBOV were not reported for nearly 15 years until a few cases were identified in 1994. This was followed by the outbreak in Zaire during 1995 in which there were 245 deaths among 316 identified cases. Ebola virus has now become endemic in many parts of Africa, with outbreaks occurring in Gabon, Sudan, Uganda, the Republic of the Congo, and the Democratic Republic of the Congo; case fatality rates range from 41% to 90%¹⁸ (Appendix).

From the first cases of MARV infection among humans in 1967, filovirus infections have been consistently linked to infected nonhuman primates or associated with caves or mines (Appendix). Often,

index cases have had suspected contact with infected animals or their products (bats, rodents, meat from apes, monkeys, or wild antelopes), but direct contact could not be proven in many of the outbreaks. During 1989, macaques originally captured in the Philippines that were housed in a primate facility in Reston, Va, began dying from acute hemorrhagic fever. The cause of the hemorrhagic fever was later identified as the EBOV-Reston strain; simian hemorrhagic fever was also isolated from additional animals in the cohort.³⁰ Subsequently, EBOV-Reston has been isolated from dying macaques imported to the United States and Italy from the Philippines in 1990, 1992, and 1996.³¹ Most recently, EBOV-Reston has been identified in pigs in the Philippines. The origin of their infection is not known at this time, nor whether increased numbers of deaths in the same porcine populations are related to the EBOV-Reston infection. It does appear that at least 1 person seroconverted against EBOV-Reston, but did not become ill, during this outbreak.² In 1994, a Swiss ethnologist survived an infection with the newly identified EBOV-Côte d'Ivoire after performing a necropsy on a dead chimpanzee from the Tai forest.³⁵ A forest worker involved in charcoal making was thought to be the index case during the EBOV outbreak in Kikwit, Zaire, in 1995.³⁷ The following year, another EBOV outbreak occurred in Gabon where most of the patients had contact with a dead chimpanzee that was consumed for food by the villagers.³⁴ During the last 2 decades, temporal associations between several other human epidemics and nonhuman primate deaths or die-offs have been detected.¹⁸ Specifically, almost all of the EBOV outbreaks in humans that occurred in the forest zone between Gabon and the Republic of the Congo during 2001 to 2003 were the result of handling of infected animal carcasses.^{18,44} However, primates are not thought to be the reservoir species because of the highly lethal nature of the disease in monkeys.

Search for Reservoir Species

Efforts to identify potential reservoir species for MARV and EBOV began shortly after the viruses were detected.⁵³ The initial MARV outbreaks in Germany and Yugoslavia were quickly associated with direct contact with specimens and cell cultures from monkeys (*Cercopithecus aethiops*) that originated from Uganda.^{17,54} The animals in question were in contact with South American finches and monkeys from Southeast Asia during transport from Africa; thus, the monkeys from Uganda could not be definitively identified as the primary source of infection.¹⁷ Nevertheless, contact with nonhuman primates, usually those with African origins, has been associated with many of the subsequent outbreaks of naturally acquired EBOV infection, which supports the hypothesis that the monkeys from Uganda encountered the original MARV reservoir prior to leaving Africa.

Because nonhuman primates, including great apes, are highly likely to die following filovirus infection,^{31,55,56} they are not generally considered long-term reservoir species but, instead, intermediate, indicator, or end hosts.⁵⁷ Extensive lists of potential reservoir spe-

cies for both filoviruses have been generated on the basis of the geographic and temporal distributions of the outbreaks^{53,58}; mammals have been the primary focus of several such efforts because of the relative ease of their capture and subsequent analysis.^{57,59,60} Searches in 1979 and 1980 in the Democratic Republic of the Congo and Cameroon were unlikely to identify the reservoir because they captured mainly rodents and bats that frequently came into contact with humans and that would be expected to transmit infection less sporadically than where the outbreaks were occurring.⁶⁰ An extensive survey of the local arthropod population began 4 months after the 1995 EBOV outbreak in Kikwit⁶¹; analysis of over 27,000 specimens representing 33 species provided no evidence either for or against arthropod transmission of EBOV. Results of additional laboratory analyses of arthropods have suggested that MARV can persist in *Aedes aegypti* mosquitoes for 3 weeks,⁵⁷ whereas EBOV-Reston is unable to replicate in *Aedes albopictus*, *Aedes taeniorhynchus*, or *Culex pipens* mosquitoes or the *Ornithodoros sonrai* soft tick.⁶²

It has been postulated that any filovirus reservoir must be a species that infrequently contacts human or nonhuman primate hosts, given the rarity of naturally occurring outbreaks.^{57,59} Despite this, bats have been associated with filovirus outbreaks since 1975, when a MARV-infected patient was associated with potential exposure to bats prior to development of clinical signs.⁶³ Since then, humans identified as index cases in filovirus outbreaks have been associated with potential exposures to bats in cotton factories,²³ caves,^{57,61} and mines.^{40,64} Early efforts to detect filoviruses in bats were unsuccessful, but were limited in the number of specimens and organs examined.^{60,65} Additionally, sample collection was frequently delayed after outbreaks until the end of the rainy season, by which time the bat population might no longer be representative of that present at the time of reservoir-host transmission.

Outbreaks of EBOV infection among humans and nonhuman primates have twice been geographically and temporally linked: first in Gabon in 2001, and again in the Democratic Republic of the Congo in 2005.⁶⁶ The coincidental nature of outbreaks among both humans and nonhuman primates in each region at these particular times was indicative of a high frequency of contact with the reservoir species. A new program of animal trapping, which focused on areas near chimpanzee and gorilla EBOV outbreaks, provided a sample group of more than 1,000 animals.⁶⁷ Of these, individual specimens from 3 species of fruit bats, *Epomops franqueti*, *Hypsignathus monstrosus*, and *Myonycteris torquata*, were positive for either EBOV-specific IgG antibodies or EBOV nucleotide sequences in liver or spleen tissues. However, EBOV-specific IgG was not detected in animals that were positive for viral nucleotide sequences, and there was a temporal association in the frequency of animals that yielded positive results for either test (ie, IgG detection or PCR assay). These findings led to the belief that animals with filovirus nucleotide sequences but no detectable virus-specific IgG antibodies were evaluated too soon after infection and had yet to develop a detectable immune response.^{67,68} Important to note, EBOV itself has not yet been isolated

from any trapped animal. The geographic range of all 3 fruit bat species encompasses areas in which previous EBOV outbreaks occurred (Figure 2). Interactions, either as physical competition for food or as predator-prey scenario, between the proposed reservoir species and potential nonhuman primate hosts have likely increased during recent years, as indicated by the increasing frequency of outbreaks. Additionally, humans living in EBOV-affected areas are known to consume raw nonhuman primate meat and also fruit bats.⁶⁷ The recent discovery of EBOV-Reston in pigs in the Philippines has already been hypothesized to be related to close proximity of fruit bats to the pig populations, although this link has yet to be proven.²

In both the United States and Italy, EBOV-Reston has been introduced to multiple quarantine facilities via cynomolgus macaques (*Macaca fascicularis*) that were obtained from a single export facility in the Philippines.^{30,31,69-74} Thus, it has been questioned whether EBOV may also be associated with a non-African reservoir species. Although only captive-bred animals are approved for export, wild-caught monkeys are often used to supplement the commercial breeding populations. This policy could allow for the introduction of an epizootic agent such as EBOV-Reston to the captive population.³⁹ An extensive survey of bats and other animals in the Philippines has not been conducted to date, and the associated reservoir species—and geographic origins—of EBOV-Reston is an area for further research.

Efforts to identify a reservoir for MARV have been hampered by the infrequency with which outbreaks

occur. Until 1998, infections were either confined to single individuals, with the occasional inclusion of their traveling companions or medical personnel, or were laboratory acquired.⁷⁵ Large outbreaks of MARV infections have occurred from 1998 to 2000 in the Democratic Republic of the Congo and in Angola in 2004 and 2005.^{40,48} The Angolan outbreak was associated with MARV only after several nosocomial infections developed, thus impeding epidemiologic investigations.⁴⁸ In contrast, the Democratic Republic of the Congo outbreak was quickly identified and rapidly associated with gold miners.⁴⁰ Multiple transmission events occurred over the course of the outbreak, and miners appeared to be the index cases in each transmission; this led to the postulation that physical contact with the reservoir species occurred inside the gold mines.⁴⁰ Mining was again implicated in a 2007 outbreak of MARV infection in Uganda,⁶⁴ thereby continuing the association of MARV with caves and mines. This led to the investigation of animals, including a large number of bats, in and around the mines,⁶⁴ and the eventual detection of MARV nucleic acid in liver and spleen tissues and MARV-specific IgG in serum obtained from cave-dwelling fruit bats (*Rousettus aegyptiacus*).⁶⁸

The ecologic niches of the identified fruit bat reservoir species overlap with that of the filoviruses. The ranges of *E franqueti*, *H monstrosus*, and *M torquata* and the range of *R aegyptiacus* encompass the geographic regions in which previous EBOV or MARV outbreaks have occurred, respectively (Figure 2). Additionally, the 3 species of fruit bats linked with EBOV are forest-

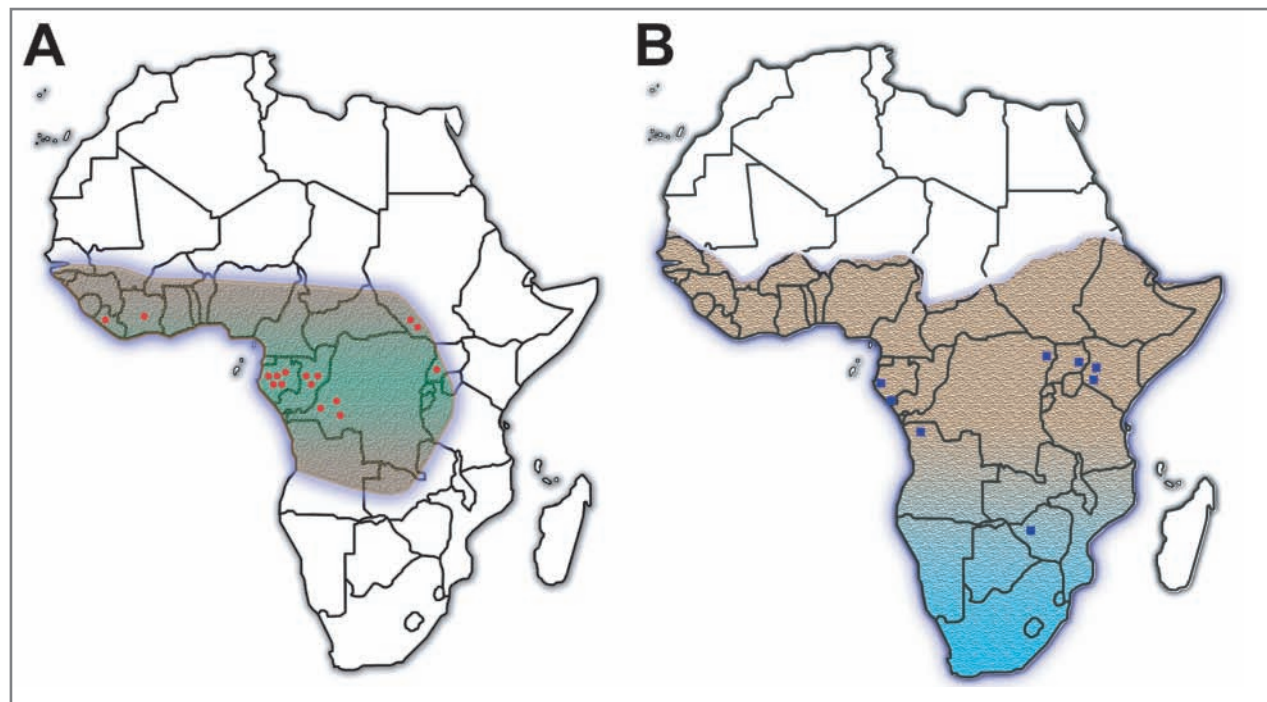


Figure 2—Maps to illustrate the geographic locations in Africa of fruit bat species implicated as possible reservoir species for EBOV and MARV with respect to outbreaks of filovirus infections. A—The geographic range (denoted by the shaded area) of 3 fruit bat species including *Epomops franqueti*, *Hypsignathus monstrosus*, and *Myonycteris torquata* that are possible reservoir species for EBOV encompasses areas of previously reported EBOV infection outbreaks (denoted by red dots). B—The geographic range (denoted by the shaded area) of the fruit bat species *Rousettus aegyptiacus* that is a possible reservoir species for MARV overlaps locations of previously reported MARV infection outbreaks (denoted by blue squares). Maps were adapted from information derived from previously published work.^{67,68}

dwellers; as such, they occupy an ecologic niche that allows for interaction with human or nonhuman primate intermediate hosts. Because of their preferred habitat, the cave-dwelling fruit bat *R. aegyptiacus* is highly likely to contact the miners and occasional spelunkers who represent most MARV index cases. Fruit bats may be only one of several reservoir species or possibly be responsible for part of the transmission cycle. In support of the latter idea, viral titers in organs of affected bats were extremely low and detectable only via PCR analysis.⁵⁰ Further supporting evidence comes from results of phylogenetic analysis, which suggest that, at least in the area near the Gabon-Congo border, EBOV-Zaire has only been associated with fruit bats since approximately 1999.⁷⁶ This recent identification of a common viral ancestor may be attributable to a so-called genetic bottleneck event (in which a large percentage of the viral population was eliminated or otherwise prevented from reproducing and only a single or small number of viral quasispecies emerged), a recent introduction of the virus to the region of interest by bats from another area, or perhaps the existence of another reservoir species.⁷⁶ It is interesting to note that the filoviruses have not been associated with South America or rain forests within southeast Asia (outside the Philippines), and this may lend further evidence toward 1 or more specific reservoir species. Filoviruses may have the ability to persist in both humans and other animals, such as rodents, nonhuman primates, antelope, pigs, and bats. This phenomenon may be more likely in reservoir species rather than in end hosts, and much more work needs to be done on this subject.^{66,77-79}

Transmission and Prevention

Zoonotic diseases are promoted and spread by activities that bring humans into close contact with wild or domesticated animals. Any contact with animals may create a new epidemic among humans; the more intense and frequent the contact, the higher the probability of disease transmission from animals to humans.⁸⁰ Regions of large biodiversity, such as rain forest regions in the Amazon, Africa, and southeast Asia, are areas in which the risk for contact between humans and animals carrying zoonotic diseases is high. Increasing encroachment into these wild habitats is one of the foremost reasons for the many rapidly emerging zoonoses. As a prime example, index cases in outbreaks of EBOV or MARV infections have been bushmeat hunters or wood gatherers who work in the dense African rain forests.^{34,37,66}

Furthermore, international trade and travel also provide new opportunities for emerging zoonotic pathogens to broadly infect human populations.⁸¹ Illegal trade of primates or other exotic animal species that are kept as pets puts humans at risk of infection with these highly lethal pathogens, but this is likely far lower than the risk of infection as a result of a burgeoning trade in illegal African bushmeat, including body parts of primates such as gorillas and chimpanzees. Secret illegal meat trade markets in cities including Paris and New York put multiple continents at risk of outbreak via zoonotic transmission, according

to the American-based Bushmeat Crisis Task Force.⁸² There are multiple recent examples of emerging diseases that have been carried through several countries and continents via air travel, including spread of EBOV by a doctor who became infected in Gabon and then traveled to South Africa.³⁸ Other viral diseases, including infections with HIV or the severe acute respiratory syndrome (SARS) coronavirus, were transformed from local problems to worldwide disasters as travel by infected humans helped to spread the viruses throughout the globe.⁸³ With global spread of these diseases, there is not only the danger of widespread human-to-human transmission, but also the possibility that these exotic diseases will find a permissive reservoir species in new locations.

Because, at least in part, the filoviruses are highly virulent and mortality rates associated with outbreaks often approach 90%, recent outbreaks of EBOV and MARV infections in Africa have been contained before the viruses were able to spread beyond the local areas. Additionally, because of the rapid time course from time of infection to death and the severe nature of the clinical signs, local, national, and international health officials have been able to react in sufficient time to institute and enforce quarantine measures. However, many patients in the 2007 EBOV outbreak in Uganda presented with mostly influenza-like symptoms, which heightened fears that patients with mild clinical signs may travel and disseminate the virus beyond the initially affected region.^{52,84} Historically, the local population and affected patients in areas of outbreaks are often highly suspicious of outsiders, especially foreigners, and residents have hidden infected individuals from local health officials. These actions by the villagers usually result in spread of the virus among family members and may endanger overall efforts to control the outbreak. Education of persons living in areas at risk for outbreaks of filovirus infections will assist in controlling spread of these dangerous diseases.⁸⁰

As part of control measures, efforts should be made to improve surveillance for filovirus outbreaks in human populations at the local, national, and international levels; these efforts should be extended to include improved surveillance of wild and domestic animals and of animal products that are considered high risk (ie, bushmeat).⁸⁵ The World Health Organization has instituted a Global Influenza Surveillance Network to monitor and evaluate new influenza strains in poultry and other livestock, thereby providing data on which recommendations for vaccine development can be made; this network proved to be useful during the recent SARS epidemic.⁸⁶ Many zoonotic diseases, including the infections with filoviruses, still remain inconsistently monitored or entirely unmonitored by public health officials.⁸⁰ Unstable political situations in several countries within Central Africa prevent active and consistent surveillance for EBOV and MARV outbreaks among nonhuman primates, as well as among humans. Furthermore, most European countries now demand mandatory testing to establish the filovirus status of imported nonhuman primates, and procedures exist worldwide to quarantine imported monkeys in an effort

to control the spread of infection. Several examples of importation of filovirus-infected macaques have been reported,^{31,39,69,87,88} including events that resulted in the 1967 outbreak of MARV infections and successive outbreaks of EBOV-Reston infections in the United States. Fortunately, these outbreaks were all contained to a reasonable extent and did not result in a widespread epidemic.

Interventions for Filoviruses

Presently, there are no medical interventions or vaccines approved for the treatment or prevention of filovirus infections in humans or other animals. In non-human primate models of lethal filovirus infections in humans, several experimental approaches have been shown to be efficacious, including treatment of the coagulopathic diathesis by use of nematode anticoagulant protein c2 (a potent inhibitor of tissue factor–initiated blood coagulation)⁸⁹ or recombinant human activated protein C (which has a broad spectrum of coagulation-modulating activity),⁹⁰ therapeutic vaccines (ie, vaccines administered after infection to reduce or arrest disease progression),^{91–93} and gene-specific antivirals.⁹⁴ Overall, approaches such as therapeutic vaccines and antivirals that directly inhibit EBOV or MARV replication appear to be the best candidates to effectively treat highly lethal filovirus infections. Lower peak viral loads are associated with better disease outcomes in both humans⁹⁵ and nonhuman primates.⁹⁴ Treatments directed against clinical signs associated with filovirus infections, such as coagulopathy or lymphocyte apoptosis, are likely to be beneficial in combination with specific antiviral treatment, when the etiologic agent is known. In general, this type of clinical sign–specific treatment will also be useful when the infectious agent has not yet been specifically identified or confirmed.

Until recently, development of vaccines against filoviruses had been a hit-or-miss effort, in part because the requirements for protective immune responses against filovirus infections are poorly understood. For instance, it remains unclear whether antibody responses, cytotoxic T-cell responses, or both are necessary to protect humans and other animals from filovirus infection.⁹⁶ The first candidate vaccines against filoviruses were based on formalin-, heat-, or irradiation-inactivated virion preparations. Partial protection of guinea pigs, rhesus macaques, and baboons following inoculation with these vaccines was reported,^{97–100} but these experimental results remain controversial because the findings have not been reproduced by other laboratories. Inactivated vaccine platforms have now been abandoned because of both production and safety issues.^{101,102} Subunit vaccine candidates based on purified filoviral protein preparations have had limited success to date.^{103,104} Partial or complete protection against homologous, but not heterologous, virus was achieved after gene-gun administration of DNA plasmids expressing the Zaire EBOV isolate (ZEBOV-May) or MARV-Mus *GP* genes into the skin of mice and guinea pigs, but provided incomplete protection to nonhuman primates.¹⁰⁵ Four vector-based approaches for vaccination against filoviruses that have efficacy in nonhuman primates have been reported including replication-incompetent Venezuelan equine en-

cephalitis virus replicons,¹⁰⁶ vesicular stomatitis Indiana virus,¹⁰⁷ replication-incompetent adenoviral vectors,¹⁰⁸ and parainfluenza-vectored vaccines.¹⁰⁹ Unfortunately, questions remain about many of the vaccine strategies applied to date, including acceptable vaccine doses; optimal routes of vaccination; requirements for booster vaccinations; duration of immunity; safety considerations including use of the vaccines in young, elderly, and immunocompromised populations; the impact of prior immunity to the vaccine vector; and the ability of these vaccine strategies to cross-protect against multiple species of EBOV and MARV. Furthermore, there is concern about potential adaptation or mutation of these new viral-based vectors in vaccinated and unvaccinated contacts because their safety profiles are not yet well established; the spread of these recombinant viruses through animal populations (both wild and domestic) is also possible. Results of recent studies^{110–119} have indicated that monovalent and multivalent FVLP-based vaccines are efficacious against EBOV and MARV infections in rodents and nonhuman primates. These FVLPs are highly immunogenic and can safely provide protection of nonhuman primates from lethal EBOV and MARV infections.^{112,113} On the basis of immunogenicity and protective efficacy of virus-like particles, as well as the known safety profiles of virus-like particle-based vaccines in general,^{118,120–123} FVLP-based vaccines are potentially one of the safest preventative treatments for use in humans and in the precious, declining nonhuman primate populations in Africa.

Overview

Until recently, only small, government-sponsored institutions were responsible for most filovirus research. Development of vaccines and research into potential antiviral drugs against zoonotic disease agents, such as the filoviruses, have not been a priority for the pharmaceutical industry. Encouragingly, progress in molecular biology and surrogate systems in the past decade have allowed further elucidation of the pathogenesis and epidemiology of these dangerous viruses, and advances in the development of effective treatments against those infections have been made.⁹⁹ Because of extensive global trade and ease of travel, a confined outbreak in Africa could easily transform into a larger, more widespread occurrence; therefore, education and awareness among local, national, and international public health officials and politicians regarding the threat of zoonotic diseases such as EBOV and MARV infections is prudent. Systematic and consistent methods for outbreak surveillance should be instituted throughout the world, including oversight on persons and wares involved in high-risk trade and travel. Certainly, current international boundaries in public health surveillance should be recognized as a major problem, and remedies for these issues should be proposed by international health agencies.

Unfortunately, the location and circumstances of the next outbreak of EBOV or MARV infection are uncertain, as evidenced by the recent discovery of EBOV-Reston in pigs in the Philippines. If either virus is transmitted from animals to humans and is then allowed to spread among humans, a potentially catastrophic situ-

ation could develop with widespread illness and death and undoubtedly worldwide panic. On the basis of knowledge to date, the filoviruses do not appear to have the biological potential for this type of doomsday scenario; however, new strains of these viruses are emerging with increasing frequency.^{40,84}

References

- Feldmann H, Jones S, Klenk HD, et al. Ebola virus: from discovery to vaccine. *Nat Rev Immunol* 2003;3:677–685.
- Cyranoski D. Ebola outbreak has experts rooting for answers. *Nature* 2009;457:364–365.
- Jeffs B. A clinical guide to viral haemorrhagic fevers: Ebola, Marburg and Lassa. *Trop Doct* 2006;36:1–4.
- Formenty P, Hatz C, Le Guenno B, et al. Human infection due to Ebola virus, subtype Cote d'Ivoire: clinical and biologic presentation. *J Infect Dis* 1999;179(suppl 1):S48–S53.
- Bwaka MA, Bonnet MJ, Calain P, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. *J Infect Dis* 1999;179(suppl 1):S1–S7.
- Stroher U, West E, Bugany H, et al. Infection and activation of monocytes by Marburg and Ebola viruses. *J Virol* 2001;75:11025–11033.
- Geisbert TW, Young HA, Jahrling PB, et al. Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis* 2003;188:1618–1629.
- Mahanty S, Hutchinson K, Agarwal S, et al. Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses. *J Immunol* 2003;170:2797–2801.
- Bosio CM, Aman MJ, Grogan C, et al. Ebola and Marburg viruses replicate in monocyte-derived dendritic cells without inducing the production of cytokines and full maturation. *J Infect Dis* 2003;188:1630–1638.
- Geisbert TW, Hensley LE, Larsen T, et al. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. *Am J Pathol* 2003;163:2347–2370.
- Gupta M, Mahanty S, Ahmed R, et al. Monocyte-derived human macrophages and peripheral blood mononuclear cells infected with ebola virus secrete MIP-1alpha and TNF-alpha and inhibit poly-IC-induced IFN-alpha in vitro. *Virology* 2001;284:20–25.
- Schnittler HJ, Feldmann H. Molecular pathogenesis of filovirus infections: role of macrophages and endothelial cells. *Curr Top Microbiol Immunol* 1999;235:175–204.
- Bradfute SB, Braun DR, Shamblin JD, et al. Lymphocyte death in a mouse model of Ebola virus infection. *J Infect Dis* 2007;196(suppl 2):S296–S304.
- Geisbert TW, Hensley LE, Gibb TR, et al. Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. *Lab Invest* 2000;80:171–186.
- Siebert R, Shu HL, Slenczka W. Detection of the “Marburg virus” in patients. *Ger Med Mon* 1968;13:521–524.
- Martini GA, Knauff HG, Schmidt HA, et al. A hitherto unknown infectious disease contracted from monkeys. “Marburg-virus” disease. *Ger Med Mon* 1968;13:457–470.
- Slenczka W, Klenk HD. Forty years of marburg virus. *J Infect Dis* 2007;196(suppl 2):S131–S135.
- Hoenen T, Groseth A, Falzarano D, et al. Ebola virus: unravelling pathogenesis to combat a deadly disease. *Trends Mol Med* 2006;12:206–215.
- Martini GA, Siebert R. Marburg virus disease. New York: Springer-Verlag Inc, 1971;230.
- The new program of the World Health Organization in medical virology. *Intervirology* 1975;6:133–149.
- Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ* 1978;56:271–293.
- Isaacson M, Sureau P, Courteille G, et al. Clinical aspects of Ebola virus disease at the Ngaliema Hospital, Kinshasa, Zaire, 1976. In: Pattyn SR, ed. *Ebola virus haemorrhagic fever*. Amsterdam: Elsevier/North-Holland Biomedical Press, 1978;15–20.
- Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. *Bull World Health Organ* 1978;56:247–270.
- Smith DH, Francis F, Simpson DIH. African haemorrhagic fever in the Southern Sudan, 1976: the clinical manifestations. In: Pattyn SR, ed. *Ebola virus haemorrhagic fever*. Amsterdam, The Netherlands: Elsevier/North-Holland Biomedical Press, 1978;21–26.
- Emond RT, Evans B, Bowen ET, et al. A case of Ebola virus infection. *Br Med J* 1977;2:541–544.
- Heymann DL, Weisfeld JS, Webb PA, et al. Ebola hemorrhagic fever: Tandala, Zaire, 1977–1978. *J Infect Dis* 1980;142:372–376.
- Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ* 1983;61:997–1003.
- Smith DH, Johnson BK, Isaacson M, et al. Marburg-virus disease in Kenya. *Lancet* 1982;1:816–820.
- Johnson ED, Johnson BK, Silverstein D, et al. Characterization of a new Marburg virus isolated from a 1987 fatal case in Kenya. *Arch Virol Suppl* 1996;11:101–114.
- Jahrling PB, Geisbert TW, Dalgard DW, et al. Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet* 1990;335:502–505.
- Rollin PE, Williams RJ, Bressler DS, et al. Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. *J Infect Dis* 1999;179(suppl 1):S108–S114.
- Hayes CG, Burans JP, Ksiazek TG, et al. Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. *Am J Trop Med Hyg* 1992;46:664–671.
- Miranda ME, White ME, Dayrit MM, et al. Seroepidemiological study of filovirus related to Ebola in the Philippines. *Lancet* 1991;337:425–426.
- Georges AJ, Leroy EM, Renaut AA, et al. Ebola hemorrhagic fever outbreaks in Gabon, 1994–1997: epidemiologic and health control issues. *J Infect Dis* 1999;179(suppl 1):S65–S75.
- Le Guenno B, Formenty P, Wyers M, et al. Isolation and partial characterisation of a new strain of Ebola virus (Erratum published in *Lancet* 2006;367:816). *Lancet* 1995;345:1271–1274.
- Le Guenno B, Formenty P, Boesch C. Ebola virus outbreaks in the Ivory Coast and Liberia, 1994–1995. *Curr Top Microbiol Immunol* 1999;235:77–84.
- Khan AS, Tshioko FK, Heymann DL, et al. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidémies à Kikwit. *J Infect Dis* 1999;179(suppl 1):S76–S86.
- Sidley P. Fears over Ebola spread as nurse dies. *BMJ* 1996;313:1351.
- Miranda ME, Ksiazek TG, Retuya TJ, et al. Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. *J Infect Dis* 1999;179(suppl 1):S115–S119.
- Bausch DG, Nichol ST, Muyembe-Tamfum JJ, et al. Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N Engl J Med* 2006;355:909–919.
- Okware SI, Omaswa FG, Zaramba S, et al. An outbreak of Ebola in Uganda. *Trop Med Int Health* 2002;7:1068–1075.
- Bitekerezo M, Kyobutungi C, Kizza R, et al. The outbreak and control of Ebola viral haemorrhagic fever in a Ugandan medical school. *Trop Doct* 2002;32:10–15.
- Nkoghe D, Formenty P, Leroy EM, et al. Multiple Ebola virus haemorrhagic fever outbreaks in Gabon, from October 2001 to April 2002 [in French]. *Bull Soc Pathol Exot* 2005;98:224–229.
- Rouquet P, Froment JM, Bermejo M, et al. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001–2003. *Emerg Infect Dis* 2005;11:283–290.
- Formenty P, Libama F, Epelboin A, et al. Outbreak of Ebola hemorrhagic fever in the Republic of the Congo, 2003: a new strategy? [in French]. *Med Trop (Mars)* 2003;63:291–295.
- Milleliri JM, Tevi-Benissan C, Baize S, et al. Epidemics of Ebola hemorrhagic fever in Gabon (1994–2002). Epidemiologic aspects and considerations on control measures [in French]. *Bull Soc Pathol Exot* 2004;97:199–205.

47. CDC. Outbreak of Marburg virus hemorrhagic fever—Angola, October 1, 2004–March 29, 2005. *MMWR Morb Mortal Wkly Rep* 2005;54:308–309.
48. Towner JS, Khristova ML, Sealy TK, et al. Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. *J Virol* 2006;80:6497–6516.
49. Outbreak news. Marburg haemorrhagic fever, Uganda. *Wkly Epidemiol Rec* 2007;82:297–298.
50. Outbreak news. Ebola virus haemorrhagic fever, Democratic Republic of the Congo—update. *Wkly Epidemiol Rec* 2007;82:345–346.
51. Outbreak news. Ebola haemorrhagic fever, Uganda—end of the outbreak. *Wkly Epidemiol Rec* 2008;83:89–90.
52. Alsop Z. Ebola outbreak in Uganda “atypical”, say experts. *Lancet* 2007;370:2085.
53. Gonzalez JP, Pourrut X, Leroy E. Ebolavirus and other filoviruses. *Curr Top Microbiol Immunol* 2007;315:363–387.
54. Luby JP, Sanders CV. Green monkey disease (“Marburg virus” disease): a new zoonosis. *Ann Intern Med* 1969;71:657–660.
55. Formenty P, Boesch C, Wyers M, et al. Ebola virus outbreak among wild chimpanzees living in a rain forest of Cote d’Ivoire. *J Infect Dis* 1999;179(suppl 1):S120–S126.
56. Walsh PD, Abernethy KA, Bermejo M, et al. Catastrophic ape decline in western equatorial Africa. *Nature* 2003;422:611–614.
57. Monath TP. Ecology of Marburg and Ebola viruses: speculations and directions for future research. *J Infect Dis* 1999;179(suppl 1):S127–S138.
58. Peterson AT, Carroll DS, Mills JN, et al. Potential mammalian filovirus reservoirs. *Emerg Infect Dis* 2004;10:2073–2081.
59. Gonzalez JP, Herbreteau V, Morvan J, et al. Ebola virus circulation in Africa: a balance between clinical expression and epidemiological silence. *Bull Soc Pathol Exot* 2005;98:210–217.
60. Breman JG, Johnson KM, van der Groen G, et al. A search for Ebola virus in animals in the Democratic Republic of the Congo and Cameroon: ecologic, virologic, and serologic surveys, 1979–1980. Ebola virus study teams. *J Infect Dis* 1999;179(suppl 1):S139–S147.
61. Reiter P, Turell M, Coleman R, et al. Field investigations of an outbreak of Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: arthropod studies. *J Infect Dis* 1999;179(suppl 1):S148–S154.
62. Turell MJ, Bressler DS, Rossi CA. Short report: lack of virus replication in arthropods after intrathoracic inoculation of Ebola Reston virus. *Am J Trop Med Hyg* 1996;55:89–90.
63. Conrad JL, Isaacson M, Smith EB, et al. Epidemiologic investigation of Marburg virus disease, Southern Africa, 1975. *Am J Trop Med Hyg* 1978;27:1210–1215.
64. Nakazibwe C. Marburg fever outbreak leads scientists to suspected disease reservoir. *Bull World Health Organ* 2007;85:654–656.
65. Leirs H, Mills JN, Krebs JW, et al. Search for the Ebola virus reservoir in Kikwit, Democratic Republic of the Congo: reflections on a vertebrate collection. *J Infect Dis* 1999;179(suppl 1):S155–S163.
66. Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. *Trends Microbiol* 2007;15:408–416.
67. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature* 2005;438:575–576.
68. Towner JS, Pourrut X, Albarino CG, et al. Marburg virus infection detected in a common African bat. *PLoS ONE* [serial online]. 2007;2:e764. Available at: www.plosone.org/home.action. Accessed Mar 30, 2009.
69. Jahrling PB, Geisbert TW, Jaax NK, et al. Experimental infection of cynomolgus macaques with Ebola-Reston filoviruses from the 1989–1990 US epizootic. *Arch Virol Suppl* 1996;11:115–134.
70. Morikawa S, Saijo M, Kurane I. Current knowledge on lower virulence of Reston Ebola virus (in French: Connaissances actuelles sur la moindre virulence du virus Ebola Reston). *Comp Immunol Microbiol Infect Dis* 2007;30:391–398.
71. CDC. Ebola-Reston virus infection among quarantined non-human primates—Texas, 1996. *MMWR Morb Mortal Wkly Rep* 1996;45:314–316.
72. Fisher-Hoch SP, Perez-Orozco GI, Jackson EL, et al. Filovirus clearance in non-human primates. *Lancet* 1992;340:451–453.
73. Ebola virus infection in imported primates—United States. *Can Dis Wkly Rep* 1990;16:17–18.
74. CDC. Ebola virus infection in imported primates—Virginia, 1989. *MMWR Morb Mortal Wkly Rep* 1989;38:831–832, 837–838.
75. Feldmann H. Marburg hemorrhagic fever—the forgotten cousin strikes. *N Engl J Med* 2006;355:866–869.
76. Biek R, Walsh PD, Leroy EM, et al. Recent common ancestry of Ebola Zaire virus found in a bat reservoir. *PLoS Pathog* [serial online]. 2006;2:e90. Available at: www.plospathogens.org/home.action. Accessed Mar 30, 2009.
77. Swanepoel R, Smit SB, Rollin PE, et al. Studies of reservoir hosts for marburg virus. *Emerg Infect Dis* 2007;13:1847–1851.
78. Gupta M, Mahanty S, Greer P, et al. Persistent infection with ebola virus under conditions of partial immunity. *J Virol* 2004;78:958–967.
79. Strong JE, Wong G, Jones SE, et al. Stimulation of Ebola virus production from persistent infection through activation of the Ras/MAPK pathway. *Proc Natl Acad Sci U S A* 2008;105:17982–17987.
80. Jones KE, Patel NG, Levy MA, et al. Global trends in emerging infectious diseases. *Nature* 2008;451:990–993.
81. Fevre EM, Bronsvoort BM, Hamilton KA, et al. Animal movements and the spread of infectious diseases. *Trends Microbiol* 2006;14:125–131.
82. Eves HE. The bushmeat trade in Africa: conflict, consensus and collaboration. In: Levigne DM, ed. *Gaining ground in pursuit of ecological sustainability*. Limerick, Ireland: International Fund for Animal Welfare, Guelph, Canada, and the University of Limerick, 2006;141–152.
83. Chevalier V, de la Rocque S, Baldet T, et al. Epidemiological processes involved in the emergence of vector-borne diseases: West Nile fever, Rift Valley fever, Japanese encephalitis and Crimean-Congo haemorrhagic fever. *Rev Sci Tech* 2004;23:535–555.
84. Towner JS, Sealy TK, Khristova ML, et al. Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog* [serial online]. 2008;4:e1000212. Available at: www.plospathogens.org/home.action. Accessed Mar 30, 2009.
85. Daszak P, Epstein JH, Kilpatrick AM, et al. Collaborative research approaches to the role of wildlife in zoonotic disease emergence. *Curr Top Microbiol Immunol* 2007;315:463–475.
86. Chretien JP, Blazes DL, Gaydos JC, et al. Experience of a global laboratory network in responding to infectious disease epidemics. *Lancet Infect Dis* 2006;6:538–540.
87. Miranda ME, Yoshikawa Y, Manalo DL, et al. Chronological and spatial analysis of the 1996 Ebola Reston virus outbreak in a monkey breeding facility in the Philippines. *Exp Anim* 2002;51:173–179.
88. DeMarcus TA, Tipple MA, Ostrowski SR. US policy for disease control among imported nonhuman primates. *J Infect Dis* 1999;179(suppl 1):S281–S282.
89. Geisbert TW, Hensley LE, Jahrling PB, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* 2003;362:1953–1958.
90. Hensley LE, Stevens EL, Yan SB, et al. Recombinant human activated protein C for the postexposure treatment of Ebola hemorrhagic fever. *J Infect Dis* 2007;196(suppl 2):S390–S399.
91. Daddario-DiCaprio KM, Geisbert TW, Stroher U, et al. Post-exposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment. *Lancet* 2006;367:1399–1404.
92. Jones SM, Stroher U, Fernando L, et al. Assessment of a vesicular stomatitis virus-based vaccine by use of the mouse model of Ebola virus hemorrhagic fever. *J Infect Dis* 2007;196(suppl 2):S404–S412.
93. Feldmann H, Jones SM, Daddario-Dicaprio KM, et al. Effective post-exposure treatment of Ebola infection. *PLoS Pathog* [serial online]. 2007;3:e2. Available at: www.plospathogens.org/home.action. Accessed Mar 30, 2009.
94. Warfield KL, Swenson DL, Olinger GG, et al. Gene-specific countermeasures against Ebola virus based on antisense phosphorodiamidate morpholino oligomers. *PLoS Pathog* [serial online]. 2006;2:e1. Available at: www.plospathogens.org/home.action. Accessed Mar 30, 2009.

95. Baize S, Leroy EM, Georges-Courbot MC, et al. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nat Med* 1999;5:423–426.
96. Mohamadzadeh M, Chen L, Olinger GG, et al. Filoviruses and the balance of innate, adaptive, and inflammatory responses. *Viral Immunol* 2006;19:602–612.
97. Chepurinov AA, Chuev Iu P, P'linkov OV, et al. The effect of some physical and chemical factors on inactivation of the Ebola virus [in Russian]. *Vopr Virusol* 1995;40:74–76.
98. Lupton HW. Inactivation of Ebola virus with 60Co irradiation. *J Infect Dis* 1981;143:291.
99. Lupton HW, Lambert RD, Bumgardner DL, et al. Inactivated vaccine for Ebola virus efficacious in guineapig model. *Lancet* 1980;2:1294–1295.
100. Mikhailov VV, Borisovich IV, Chernikova NK, et al. The evaluation in hamadryas baboons of the possibility for the specific prevention of Ebola fever [in Russian]. *Vopr Virusol* 1994;39:82–84.
101. Geisbert TW, Pushko P, Anderson K, et al. Evaluation in nonhuman primates of vaccines against Ebola virus. *Emerg Infect Dis* 2002;8:503–507.
102. Reed DS, Mohamadzadeh M. Status and challenges of filovirus vaccines. *Vaccine* 2007;25:1923–1934.
103. Agafonov AP, Ignat'ev GM, Kuz'min VA, et al. The immunogenic properties of Marburg virus proteins [in Russian]. *Vopr Virusol* 1992;37:58–61.
104. Hevey M, Negley D, Geisbert J, et al. Antigenicity and vaccine potential of Marburg virus glycoprotein expressed by baculovirus recombinants. *Virology* 1997;239:206–216.
105. Vanderzanden L, Bray M, Fuller D, et al. DNA vaccines expressing either the GP or NP genes of Ebola virus protect mice from lethal challenge. *Virology* 1998;246:134–144.
106. Hevey M, Negley D, Pushko P, et al. Marburg virus vaccines based upon alphavirus replicons protect guinea pigs and nonhuman primates. *Virology* 1998;251:28–37.
107. Jones SM, Feldmann H, Stroher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med* 2005;11:786–790.
108. Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature* 2003;424:681–684.
109. Bukreyev A, Rollin PE, Tate MK, et al. Successful topical respiratory tract immunization of primates against Ebola virus. *J Virol* 2007;81:6379–6388.
110. Swenson DL, Warfield KL, Kuehl K, et al. Generation of Marburg virus-like particles by co-expression of glycoprotein and matrix protein. *FEMS Immunol Med Microbiol* 2004;40:27–31.
111. Swenson DL, Warfield KL, Larsen T, et al. Monovalent virus-like particle vaccine protects guinea pigs and nonhuman primates against infection with multiple Marburg viruses. *Expert Rev Vaccines* 2008;7:417–429.
112. Swenson DL, Warfield KL, Negley DL, et al. Virus-like particles exhibit potential as a pan-filovirus vaccine for both Ebola and Marburg viral infections. *Vaccine* 2005;23:3033–3042.
113. Warfield KL, Swenson DL, Olinger GG, et al. Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *J Infect Dis* 2007;196(suppl 2):S430–S437.
114. Warfield KL, Bosio CM, Welcher BC, et al. Ebola virus-like particles protect from lethal Ebola virus infection. *Proc Natl Acad Sci U S A* 2003;100:15889–15894.
115. Warfield KL, Olinger G, Deal EM, et al. Induction of humoral and CD8+ T cell responses are required for protection against lethal Ebola virus infection. *J Immunol* 2005;175:1184–1191.
116. Warfield KL, Perkins JG, Swenson DL, et al. Role of natural killer cells in innate protection against lethal ebola virus infection. *J Exp Med* 2004;200:169–179.
117. Warfield KL, Posten NA, Swenson DL, et al. Filovirus-like particles produced in insect cells: immunogenicity and protection in rodents. *J Infect Dis* 2007;196(suppl 2):S421–S429.
118. Warfield KL, Swenson DL, Demmin G, et al. Filovirus-like particles as vaccines and discovery tools. *Expert Rev Vaccines* 2005;4:429–440.
119. Warfield KL, Swenson DL, Negley DL, et al. Marburg virus-like particles protect guinea pigs from lethal Marburg virus infection. *Vaccine* 2004;22:3495–3502.
120. Noad R, Roy P. Virus-like particles as immunogens. *Trends Microbiol* 2003;11:438–444.
121. Stanley MA. Human papillomavirus vaccines. *Curr Opin Mol Ther* 2002;4:15–22.
122. Harro CD, Pang YY, Roden RB, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst* 2001;93:284–292.
123. Martin SJ, Vyakarnam A, Cheingsong-Popov R, et al. Immunization of human HIV-seronegative volunteers with recombinant p17/p24:Ty virus-like particles elicits HIV-1 p24-specific cellular and humoral immune responses. *AIDS* 1993;7:1315–1323.

Continued on next page.

Appendix

Summary of reported outbreaks of filovirus infections and the association of affected humans with exposure to other animals or to caves or mines.

Year	Virus isolate	Location	No. of reported human cases (case fatality rate [%])	Association of infected humans with other animals or cave or mine environments	Reference No.
1967	MARV-Popp/Ci67	Germany and Yugoslavia	31 (21)	Laboratory workers in contact with African green monkeys imported from Uganda.	19
1975	MARV	Johannesburg, South Africa	3 (33)	Affected person had returned from Zimbabwe; potential exposure to bats during trip. Traveling companion and an attending nurse were subsequently infected.	20
1976	EBOV-Zaire	Yambuku region, Zaire (now DRC)	318 (88)		21, 22
1976	EBOV-Sudan	Nzara and Maridi regions, Sudan	284 (53)	Evidence of bats at cotton factory (work location of index case).	23, 24
1976	EBOV-Sudan	England	1 (0)		25
1977	EBOV-Zaire	Tandala village, Zaire (now DRC)	1 (100)		26
1979	EBOV-Sudan	Nzara area, Sudan	34 (65)	Evidence of bats at cotton factory (work location of index case).	27
1980	MARV	Kitum Cave on Mount Elgon, Kenya	2 (50)	Index case visited cave.	28
1987	MARV	Kitum Cave on Mount Elgon, Kenya	1 (100)	Index case visited cave.	29
1989	EBOV-Reston	United States	0 (0)	Nonhuman primates imported from the Philippines.	30
1990	EBOV-Reston	United States	0 (0)	Nonhuman primates imported from the Philippines.	31
1989–1990	EBOV-Reston	Philippines	0 (0)	High mortality rate among wild-caught macaques.	32, 33
1992	EBOV-Reston	Sienna, Italy	0 (0)	Nonhuman primates imported from the Philippines.	32, 33
1994	EBOV-Zaire	Gabon	49 (59)	Outbreak near gold-mining camps (possible association with bats or other cave-dwelling animals).	34
1994	EBOV-Ivory Coast	Tai Forest, Ivory Coast	1 (0)	Infected person participated in necropsy of wild chimp.	35, 36
1995	EBOV-Ivory Coast	Liberia	1 (0)	Retrospective identification from Liberian refugee.	36
1995	EBOV-Zaire	Kikwit area, Zaire (now DRC)	315 (81)	Forest worker involved in charcoal making thought to be the index case.	37
1996	EBOV-Zaire	Mayibout area, Gabon	37 (57)	Chimp found dead; meat had been eaten.	34
1996–1997	EBOV-Zaire	Booue area, Gabon	60 (74)	Dead chimp found in forest (not primary contact case).	34
1996	EBOV-Zaire	South Africa	2 (50)	Animal association unknown; introduced by ill patient from Gabon.	38
1996	EBOV-Reston	United States	0 (0)	Nonhuman primates imported from the Philippines.	31
1996	EBOV-Reston	Philippines	0 (0)	Nonhuman primates imported from the Philippines.	39
1998–2000	MARV	Durba, DRC (formerly Zaire)	154 (83)	Working in a local mine was identified as a risk factor.	40
2000–2001	EBOV-Sudan	Gulu, Masindi, and Mbarara districts, Uganda	425 (53)		41
2001–2002	EBOV-Zaire	Ogooue-Invindo province, Gabon	65 (82)	Infected persons had contact with dead or butchered wildlife; concurrent outbreaks in wild gorillas, chimpanzees, and duiker.	42–44
2001–2002	EBOV-Zaire	Mekambo, Mbomo, and Kelle districts, Republic of the Congo	57 (75)	Infected persons had contact with dead or butchered wildlife; concurrent outbreaks in wild gorillas, chimpanzees, and duiker.	43, 44
2002–2003	EBOV-Zaire	Mbomo and Kelle districts, Republic of the Congo	143 (89)	Infected persons had contact with dead or butchered wildlife; concurrent outbreaks in wild gorillas, chimpanzees, and duiker.	44, 45
2003	EBOV-Zaire	Mbomo and Mbandza districts, Republic of the Congo	35 (83)	Infected persons had contact with dead or butchered wildlife; concurrent outbreaks in wild gorillas, chimpanzees, and duiker.	44, 46
2004	EBOV-Sudan	Yambio County, Sudan	17 (41)		47
2004–2005	MARV-Angola	Uige Province, Angola	252 (90)		48
2007	MARV	Kamwenge District, Uganda	2 (50)	Infected persons worked in same mine (2-mo interval between infections).	49
2007	EBOV-Uganda	Bundibugyo District, Uganda	Preliminary estimates of 149 (approx 25)		50–52

DRC = Democratic Republic of the Congo.